in some instances, as indicated by the presence of multiple budded colonies which could not have stood erect independently (Fig. 1A). It is probable that growth in many instances was influenced by the encroachment of sediment on the colony surface, resulting in the concentration of steeply grown and multiply budded colonies in areas of relatively rapid sedimentation. This relationship is evidenced in Missouri, where steeply grown and rejuvenescent forms prevail, and the rock is characterized by an abundance of poorly sorted clastic material, indicating relatively rapid deposition. Other variation in growth rate may have resulted from seasonal changes, storm turbulence, or other environmental parameters.

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References and Notes

1. Denotes North American classification. The Lower-Middle boundary corresponds to the Canadian-Champlainian Series boundary, and is currently defined within the *Didymograptus bifidus* graptolite zone, and between the "*Prioniodus*" *evae* and *Eoplacognathus variabilis* conodont zones. This boundary corresponds to the *D. nitidus-Isograptus gibberulus* boundary within the Arenig Series of the British succession. Reports of "Lower" Ordovician bryozoans from Estonia (B_{II} interval) are from beds equivalent to the *I. gibberulus* zone or younger [L. E. Fahreaus, *Bull. Can. Pet. Geol.* **26**, 5 (1977)].
2. *Marcusodictyon*, R. S. Bassler (*5*); *Archaeotrypa* [M. A. Fritz, *J. Paleontol.* **21**, 434 (1947)]; *Ceramopora*? [J. P. Ross, *Okla. Geol. Notes* **26**, 218 (1966)].
3. G. G. Astrova, *Tr. Paleontol. Inst. Akad. Nauk SSSR* **106** (1965).
4. The Black Rock unit has alternately been considered as a formation, a member of the Smithville formation (*6*), and with the Smithville unit, as lithosomes of the Powell formation (*7*). Neither it nor the Smithville carry formal stratigraphic unit status.
5. R. S. Bassler, *U.S. Natl. Mus. Bull. 77* (1911).
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7. W. J. Hedden, dissertation, University of Missouri, Rolla, (1976); *Diss. Abstr. B*, 4367-B (1977).
8. The latest stage in the Canadian Series. Approximates the upper Latorp Stage of the Baltoscandia area.
9. They are characterized by recrystallized microstructure, thin-walled zooecial tubes, mesopores, obscure acanthoporoids, and a subtle or missing endozone to exozone transition.
10. G. G. Astrova, *Paleontol. Zh.* **2**, 22 (1964).
11. Like other species of *Dianulites*. the walls are thin, and show no apparent thickening in the exozone. The tubes grew parallel, and are polygonal in transverse section (Fig. 1B), with distinct polymorphism between smaller (0.15 mm) mesozooecia and larger (0.25 mm) autozooecia. In longitudinal section (Fig. 1D) the mesozooecia are elevated several diaphragm levels above adjacent, depressed autozooecia. Maculae appear as dense spots on the colony surface (Fig. 1C), and consist of clusters of mesozooecia.
12. Otherwise known to occur in only one other bryozoan, *Dianulites fastigiatus* from B_{II}-B_{III} interval of Estonia.
13. I thank D. B. Blake, R. S. Boardman, R. J. Cuffey, O. L. Karklins, M. T. Jollie, F. K. McKinney, J. P. Ross, G. J. Retallack, and M. P. Weiss for reviewing this report and M. F. Fix, S. H. Frost, J. Utgaard, O. A. Wise, and E. L. Yochelson for help and discussion.

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Collagenase Immunolocalization in Cultures of Rheumatoid Synovial Cells

Abstract. *Cultures of rheumatoid synovial cells that have been enzymatically dissociated and are adherent to a culture vessel are morphologically heterogeneous. When these cells are cultured on a collagenous substrate for 2 to 6 days at 37°C in serum-free medium, they produce collagenase. A monospecific antibody to human collagenase has localized the enzyme extracellularly around cytoplasmic extensions of dendritic cells and intracellularly within a few macrophage-like and fibroblast-like cells.*

Rheumatoid synovial collagenase is a metallo-enzyme with basic charge properties and a molecular weight of 33,000 (*1*). The enzyme is inhibited by Ca^{2+}-chelating agents, thiol reagents (*2*), and by the serum components α_2-macroglobulin and β_1-anticollagenase (*3*). A monospecific immunoglobulin G (IgG) antibody to active human collagenase, prepared in sheep (*4*), has been used to identify the immunoreactive enzyme at the cartilage-pannus junction in diseased articular tissue (*5*). These studies confirmed the association of collagenase with joint destruction in rheumatoid arthritis in vivo (*2*, *6*).

Rheumatoid synovial cells that have been dissociated enzymatically from synovial tissue and are adherent to culture vessel surfaces are heterogenous (*7*). Included among these are cells that morphologically resemble fibroblasts, large rounded cells, and unusual "dendritic" or "stellate" cells with rounded-up cytoplasm and long radial extensions. These cells have a slow doubling time, rarely reach confluence, and make little lysozyme; however, they synthesize and release collagenase in quantities up to 100-fold that reported for rabbit synovial fibroblasts or stimulated macrophages (*8*).

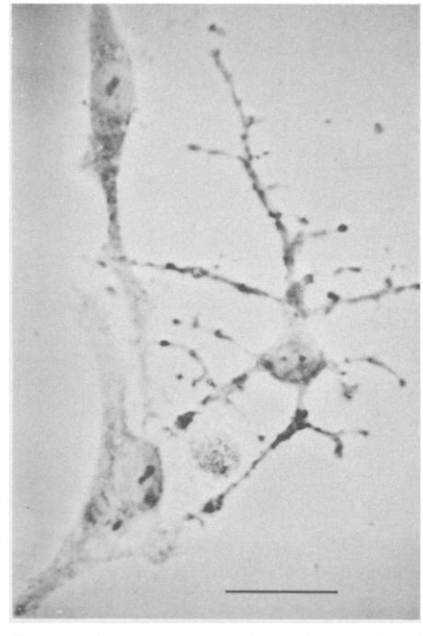

Fig. 1. Phase-contrast photomicrograph of isolated adherent rheumatoid synovial cells stained with toluidine blue. The dendritic cell, characterized by branched cytoplasmic extensions, contains many spherical phase-dense cytoplasmic granules which, from Janus green staining, indicate mitochondria (*9*). Bar represents 20 μm.

A typical dendritic cell and two fibroblast-like cells, which comprise more than 80 percent of the total cell population of such preparations, are shown in Fig. 1. Histological studies with Janus green and toluidine blue have shown that most of these dendritic cells contain many dense bodies packed into the cell processes, and have a stained appearance similar to that of large mitochondria (*9*). The failure of these dendritic cells to phagocytize latex particles suggests that they are functionally different from the fibroblast- and macrophage-like cells (*10*).

Dissociated rheumatoid synovial cells were dispersed in Dulbecco's modified Eagle's medium (DMEM) with 10 percent fetal calf serum, and grown on cover slips coated with thin films of thermally reconstituted collagen that were dried at 37°C (*7*). After 24 hours, the cells were washed free of serum-containing culture medium, and the cultures were continued in DMEM with 0.2 percent lactalbumin hydrolysate. After an additional 48- or 72- hour incubation, the collagen-coated cover slips were fixed for 30 seconds in 80 percent ethanol and air-dried. Indirect immunofluorescence, with monospecific sheep antibody to synovial collagenase and fluorescein isothiocyanate (FITC) labeled rabbit antibody to sheep immunoglobulin G (*5*), was used to localize collagenase in these cultures (Fig. 2).

Immunoreactive collagenase was associated chiefly with dendritic cells. The FITC fluorescence located the enzyme around (and probably within) the cytoplasmic extensions, as if it had been bound or trapped by the collagen substratum. However, only a proportion of the dendritic cells were associated with extracellular enzyme. Some fibroblast-

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like and macrophage-like cells showed intense intracellular localization of collagenase in the perinuclear region (*10*), although no extracellular enzyme was detected around them.

These data suggest that dendritic cells in vitro are important collagenase-producers, releasing enzyme in a form readily detected by antibody. The finding of only intracellular enzyme in fibroblast-like cells suggests that, if these cells secrete enzyme, it is either nonimmunoreactive or does not bind to the underlying collagen.

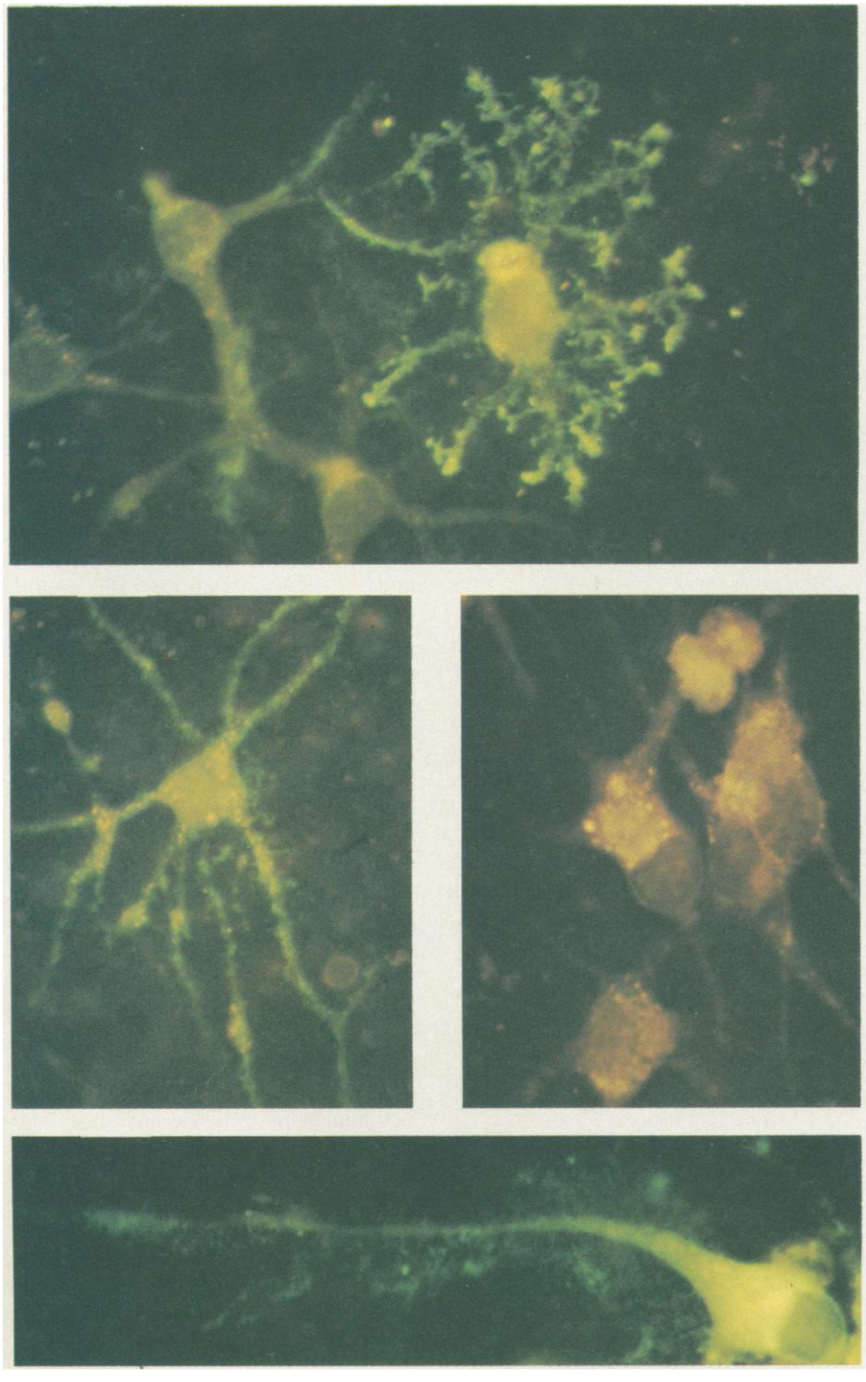

Fig. 2. Immunohistochemical demonstration of collagenase produced by dendritic rheumatoid synovial cells (× 900). The heterogeneous population of dissociated synovial cells, attached to collagen-coated glass cover slips, were cultured in serum-free DMEM supplemented with 0.2 percent lactalbumin hydrolysate for 72 hours at 37°C (*7*). FITC-immunofluorescent studies with monospecific antibody to synovial collagenase (*5*) showed extracellular localization of enzyme associated only with the dendritic cells. Eriochrome black (Difco, 1:10 dilution, 1 minute at 21°C) was used to counterstain the cytoplasm to orange-red. (Top) Dendritic cell demonstrating positive FITC-fluorescence. Note lack of immunoreactive enzyme in adjacent dendritic cells. (Left center) Dendritic cell with extracellular enzyme associated with cytoplasmic extensions. (Right center) Cells treated with nonimmune sheep immunoglobulin G as a control (*5*) and counterstained with eriochrome black. (Bottom) Collagenase localized extracellularly around cytoplasmic extension of a dendritic cell.

Monolayer synovial cell cultures, maintained in serum-free culture medium supplemented with lactalbumin hydrolysate, produce collagenase in both active and latent forms; however, the latent form predominates during the first few days of culture (*7*). Latent collagenase from these cultures reacted with antibody to collagenase by immunodiffusion, but it produced a weaker precipitin response than the equivalent amount of activated enzyme (*10*). Previous studies (*11*) had indicated that the latent enzyme bound to collagen fibrils less than half as well as did active enzyme at room temperature. To compare immunofluorescence of the two forms of collagenase, 1.5 units per milliliter of active enzyme (1 unit degrades 1 μg collagen fibrils per minute at 37°C) was added for 10 minutes at 21°C to an untreated 8-μm cryostat section of synovial tissue. An equivalent activity of the latent enzyme, determined through activation, was added to a similar section for 5 hours. Sections were then washed briefly in DMEM and were fixed with ethanol. Subsequent antibody treatment showed little fluorescence in the latent enzyme-treated tissue section, whereas intense fluorescence was demonstrated in the sections treated with active enzyme. This could be explained either by the failure of latent enzyme to bind the collagen of the tissue, or by its poor immunoreactivity after binding to collagen. These observations supported the view that extracellular fluorescence in the synovial cell cultures was due largely to active enzyme bound to the collagen substratum. This is further supported by the failure to detect positive immunofluorescence during the early stages of culture, even though control cultures showed the presence of latent enzyme in the medium.

Recent studies have suggested that an effective inhibitor of active collagenase is produced by rheumatoid synovial cells in culture (*12*). As with the enzyme-inhibitor complex found in cultures of various rabbit tissues (*13*), an excess of inhibitor may be produced during the early stages of culture; another possibility is that different cells synthesize variable amounts of active enzyme and inhibitor.

One interpretation of our observations is that the dendritic cells produce great quantities of active enzyme; during the later stages of culture, there is more enzyme than free inhibitor produced by the heterogenous cell population, and it appears as collagen-bound enzyme around the site of secretion. Although the restriction of fluorescence to the close proximity of the cells suggests a relative-

ly temporary deposition rather than an accumulation of enzyme over 2 or 3 days, it is unknown for how long the immunoreactive enzyme has been present in the observed locations.

These data suggest that collagenase is released by the long dendritic processes (Fig. 2), and that the large number of dense bodies found in these extensions (Fig. 1) may be related to enzyme storage or release. Immuno-ultrastructural studies may help to elucidate the mechanism of collagenase secretion or exocytosis in these cytoplasmic processes, whether the perinuclear region of cells demonstrating FITC-fluorescence represents Golgi apparatus, and how the enzyme is transported to the site of secretion.

It is not known whether the dendritic cell observed in vitro in monolayer cultures has the same morphology in vivo, or what the origin or distribution of this cell is in rheumatoid synovial tissue. The dendritic cells may be synovial cells transformed in morphology and function by components of chronic inflammation, much as bone cells may be transformed in vitro from a spherical to a stellate appearance by certain hormones and cyclic nucleotides (*14*). Since we now know that collagenase is produced at sites of cartilage resorption in vivo (*5*) and that the dendritic cell has the ability to produce large quantities of this enzyme in vitro, further studies on the role of this cell in the pathophysiology of the rheumatoid joint are essential.

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Different Actions of Anticonvulsant and Anesthetic Barbiturates Revealed by Use of Cultured Mammalian Neurons

Abstract. *Barbiturate anesthetics, but not anticonvulsants, abolish the spontaneous activity of cultured spinal cord neurons; directly increase membrane conductance, an effect which is suppressed by the γ-aminobutyric acid (GABA) antagonists picrotoxin and penicillin; and are more potent than anticonvulsants in augmenting GABA and depressing glutamate responses. Barbiturate anticonvulsants abolish picrotoxin-induced convulsive activity. These results indicate qualitative and quantitative differences between anesthetic and anticonvulsant barbiturates, which may explain their different clinical effects.*

Barbiturates are used therapeutically for their varied pharmacological effects. Phenobarbital is an effective anticonvulsant at clinical doses that produce minimal sedation, while pentobarbital is commonly used as an anesthetic because of its dominant sedative action. Such disparate pharmacological effects may reflect different mechanisms for attenuating central nervous system (CNS) excitability, although such a comparative study has not been performed. Pentobarbital has multiple effects, including (i) enhanced presynaptic inhibition of sensory afferents (*1*), possibly mediated by γ-aminobutyric acid (GABA) (*2*); (ii) depression of transmitter release at sensory afferent synapses into the CNS (*3*); (iii) enhanced transmitter release at peripheral neuromuscular junctions (*4*); (iv) depression of excitatory synaptic transmission at a variety of peripheral and central synapses (*5–7*); (v) enhancement of GABA-mediated postsynaptic inhibition (*7–10*); and (vi) direct effects on sensory afferents and spinal cord neurons (*1, 7, 9, 10*) that are abolished by the GABA antagonist picrotoxin (*1, 9*). Thus, barbiturate anesthesia is thought to result from the combined effect of enhanced inhibition and diminished excitation, producing a net decrease in neuronal excitability.

We have compared the actions of anesthetic and anticonvulsant barbiturates using a mammalian tissue culture system. We have found that (i) anesthetics, but not anticonvulsants, abolish all spontaneous synaptic activity at therapeutic concentrations, and anticonvulsants abolish picrotoxin-induced paroxysmal events; (ii) anesthetics, but not anticonvulsants, directly increase membrane conductance, an effect which can be antagonized by the GABA-specific antagonists picrotoxin and penicillin (*11, 12*); (iii) anesthetics, but not anticonvulsants, substantially prolong GABA responses; and (iv) both classes of barbiturates enhance postsynaptic GABA responses and antagonize postsynaptic glutamate responses, although anesthetics are two to three times more potent than anticonvulsants. These results indicate qualitative and quantitative differences in the pharmacological actions of anticonvulsant and anesthetic barbiturates, which may explain their different clinical effects.

Spinal cords were dissected from 12-to 14-day-old mouse embryos, mechanically dissociated, and grown in tissue culture for 5 to 13 weeks before electrophysiological study (*13*). Recordings were made on a modified stage of an inverted phase microscope. Large, multipolar spinal cord neurons were penetrated under direct vision with glass micropipettes (25 to 50 megohms filled with 4*M* potassium acetate or 3*M* KCl. The legends to Figs. 1 and 2 contain additional details of methodology.

Spontaneous activity in these cultures (Fig. 1A1) was characterized by abundant excitatory and inhibitory postsynaptic potentials, random firing of action potentials with varying frequency, and occasional short duration (< 0.5 sec-

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